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Characterization of *Artemia* from different localities in Tunisia with regard to their use in local aquaculture

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Abstract

Artemia cysts have been collected from different saltworks and natural salt lakes in Tunisia. The cyst material was processed and used for the following characterization analyses : cyst and naupliar biometrics, cyst hatching characteristics, fatty acid pattern of the nauplii, nutritional value of the nauplii for mysid shrimp, naupliar growth rate and temperature resistance, mode of reproduction, and characterization of sibling species. The cross-breeding tests and cyst biometrics reveal that only *Artemia tunisiana* occurs in Tunisia. Although quality improvements may be expected through improved harvesting, Tunisian *Artemia* have acceptable hatching characteristics and are a good food for mysid shrimp. Small variations in hatching quality observed between cyst samples harvested from the same locality, might indicate fluctuations in the environmental conditions prior to the cyst harvest. Naupliar growth rate is high in most strains but naupliar resistance to high temperatures is limited.

Introduction

In future years coastal aquaculture in Tunisia is expected to expand rapidly with the production of sea bass *Dicentrarchus labrax*, sea bream *Sparus aurata*, and *Penaeus* sp. Since Tunisian fish and shrimp hatcheries will need substantial quantities of *Artemia*, and since several natural *Artemia* habitats are known in Tunisia (Persoone and Sorgeloos, 1980 ; Vanhaecke *et al.*, 1987), a survey was set up to sample brine shrimp in order to characterize their potential value in marine fish and shrimp farming. Cysts were processed and submitted to standard laboratory tests to define the taxonomic classification of Tunisian *Artemia*, their suitability for local culture, and the economical and nutritional quality of their cysts.

Material and methods

Cyst samples

Cysts were collected from three salt lakes, Sebkret el Kourzia, Sebkret Sidi el Hani, and Sebkret mta Moknine, and from the solar saltworks near Sfax, Bekalta, and Mégrine (Fig. 1). Sebkret el Kourzia and Sebkret Sidi el Hani, both inland lakes, were dry at the time of sampling but cysts were found embedded in the salt crusts. At Sebkret mta Moknine, *Artemia* cysts were collected along the shore of the lake. In the Mégrine and Bekalta salterns, cysts were sampled from the shore of evaporation ponds with a salinity of 152 and 200-230 ‰ respectively. Two samples were taken in Sfax, *i.e.* from the shore of a 117 ‰ S evaporation pond (Sfax 117) and scooped from the water in a 218 ‰ S brine collector pond (Sfax 218).

All samples were processed at the Artemia Reference Center. Cysts were cleaned by means of the bi-flotation technique as described by Sorgeloos *et al.* (1978) and, exception made for the Sfax samples, dried in a fluidized bed dryer at a temperature of 35 °C. The Sfax material was dried on a 120 µm screen in a temperature-controlled room (35 °C) under continuous ventilation. The lake samples, containing considerable amounts of salt and impurities, produced only limited amounts of full cysts, especially the Kourzia sample, restricting the characterization of the latter to the analysis of the reproductive mode.

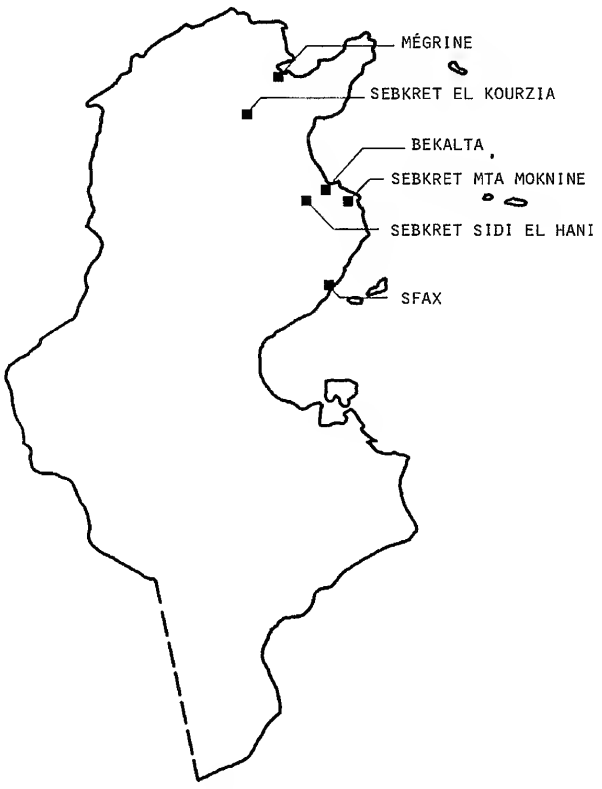

Fig. 1. Location of the Tunisian *Artemia* sources studied.

ARTEMIA CULTURE TESTS

All *Artemia* culture tests were carried out in 35 ‰ S artificial seawater (formula in Sorgeloos *et al.*, 1983). Once a day the larvae were fed *Dunaliella viridis* cells according to optimal feeding regimes as determined by Vanhaecke (1983).

For the analysis of the reproduction mode *Artemia* larvae were cultured at 25 °C in cylindro-conical tubes each containing 150 freshly-hatched nauplii in 300 ml seawater. When sexual differentiation was clearly pronounced, survival and sex ratio were determined and the animals transferred to test tubes, each holding 10 females in 15 ml seawater or 10 couples in 25 ml seawater. Both couple-cultures and all-female cultures, were regularly checked for viable offspring.

Artemia sibling species were defined for Sfax and Mégrine brine shrimp in cross-breeding tests performed following procedures outlined by Tackaert *et al.* (1987) with *Artemia* from San Francisco Bay (California-USA) and Larnaca Lake (Cyprus) as reference material for respectively *A. franciscana* (Bowen *et al.*, 1978) and *A. tunisiana* (Vanhaecke *et al.*, 1987).

Larval growth and survival of Mégrine, Bekalta, Sfax, Moknine, and Sidi el Hani *Artemia* were determined in a standard culture test as outlined by Vanhaecke and Sorgeloos (1980a). Growth of the nauplii was expressed as percent of the growth recorded for the San Francisco Bay strain 288-2596 which was included as an internal standard.

The response of Tunisian *Artemia* to high temperatures was studied following the test procedure of Vanhaecke *et al.* (1984), but omitting the different salinity regimes. Mégrine, Bekalta, and Sfax *Artemia* were cultured at a salinity of 35 ‰ and temperatures of 30 °C and 34 °C ; *Artemia* from Moknine was cultured at 30 °C. *Artemia* from Great Salt Lake (1977), known to have a high temperature tolerance (Vanhaecke *et al.*, 1984), was selected as a reference.

HATCHING TESTS

Hatching tests were carried out in 35 ‰ S artificial seawater (formula of Dietrich and Kalle, 1957) at an incubation temperature of 25 °C ± 0.5 °C under continuous illumination of 1 000 lux. The hatching vessels, were cylindro-conical glass tubes and cysts were kept in suspension by gentle air bubling from the cone of the tube. Hatching percentage, hatching efficiency, and hatching rate (and synchrony) were analyzed as outlined by Bruggeman *et al.* (1979), Sorgeloos *et al.* (1978), and Vanhaecke and Sorgeloos (1982) respectively. Hatching efficiency is expressed as the number of nauplii hatched out of 1 g dry cyst product after 48 h incubation ; hatching rate is expressed as number of hours of incubation needed to reach the time of first appearance of nauplii and 50 % and 90 % of the maximal hatching value.

CYSTS AND NAUPLII BIOMETRICS

Diameter, volume, and chorion thickness of the cysts and length of the nauplii were analyzed for the Mégrine, Bekalta, and Sfax 218 samples, while cysts from Moknine and Sfax 117 were analyzed respectively for the first two and first three biometrical parameters above. Size analysis of the cysts was performed on both untreated and decapsulated (Bruggeman *et al.*, 1980) fully-hydrated cysts using Coulter Counter equipment (Vanhaecke *et al.*, 1980). Analysis of the naupliar length was performed according to the method of Vanhaecke and Sorgeloos (1980b) and Vanhaecke (1983). Individual dry weight of the nauplii was calculated from the equation based on cyst volume (Vanhaecke, 1983).

Fatty acid analysis

The fatty acid profile was determined for freshly-hatched nauplii from the Mégrine and Sfax 218 samples. After homogenization with an ultrasonic homogenizer, lipid extraction, saponification, and esterification were done according to the procedure described by Schauer and Simpson (1978). Fatty acid methyl esters were injected on a capillary column (25 m fused silica, 0.32 mm ID ; liquid phase : Silar 10 C ; film thickness, 0.3 μm) installed on a Carlo Erba Fractovap 2330 gas chromatograph. Operating conditions were as follows : solid injector ; hydrogen as carrier gas at a flow rate of 1.9 ml/min ; FID detection ; oven temperature program : 154 °C-200 °C at 1.5 °C/min. Peak identification and quantification was done with a calibrated plotter-integrator (Hewlett Packard 3390A). The internal standard procedure with 20:2ω6 was used for quantitative analysis.

Culture test with mysid shrimp

The culture test with the marine mysid *Mysidopsis bahia* was performed as outlined by Léger *et al.* (1987). Mysid juveniles were fed daily with freshly-hatched nauplii. Survival was registered daily and at the end of the experiment growth and reproductive characteristics of the mysids were examined. Nauplii hatched from Reference *Artemia* Cysts and Great Salt Lake North Arm cysts were included in the culture experiment as positive and negative controls, respectively.

Data analysis

Data from the standard *Artemia* growth test, the temperature resistance experiments, and the bioassay with mysid shrimp were treated statistically in a one-way analysis of variance (Sokal and Rohlf, 1969). Duncan's multiple range test was used to determine significant differences among means (Goodnight, 1979) and the survival data were normalized through an arcsin $\sqrt{\%}$ transformation prior to analysis (Snedecor and Cochran, 1967).

Results and discussion

All Tunisian *Artemia* tested so far are bisexual (Table I) : sex ratios are high and females cultured in the absence of males produced non-viable eggs only. Since the crossbreeding tests between either Sfax or Mégrine *Artemia* with Larnaca (Cyprus) brine shrimp yielded viable F1 offspring, whereas Sfax and San Francisco Bay matings were sterile, Sfax and Mégrine *Artemia* can be classified within the *Artemia tunisiana* sibling species complex.

Table I

Sex-ratio and reproductive mode in *Artemia* from Tunisia

	Mégrine	Bekalta	Sfax 218	Moknine	Sidi el Hani	Kourzia	Ariana[1]
Sex ratio (male/female)	1.38	1.5	1.0	1.0	0.9	–[2]	–
Reproductive mode	B[3]	B	B	B	B	B	B

[1] After Clark and Bowen (1976).

[2] No data available.

[3] Bisexual.

With the exception of the strain from Sfax, the cyst diameters of the Tunisian populations (Table II) resemble those of the other Old World bisexual populations (Léger *et al.*, 1986) ; *i.e.* significantly larger than the mean cyst diameter of *Artemia franciscana* strains, thus providing further evidence for their designation as *Artemia tunisiana.* Despite being a representative of the *Artemia tunisiana* sibling species as proven by their reproductive compatibility with the *Artemia tunisiana* strain from Larnaca, the biometrical characteristics of Sfax *Artemia* are aberrant since their cyst diameter is significantly smaller than the mean value found for the other *Artemia tunisiana* strains, while their chorion thickness lies between the mean values obtained for *Artemia tunisiana* and *Artemia franciscana.*

TABLE II

Diameter and chorion thickness (in µm) of *Artemia* cysts form Tunisia

	Mégrine	Bekalta	Sfax 218	Sfax 117	Moknine
Diameter of untreated cysts	258.8	251.6	235.4	239.7	252.6
standard deviation	(14.9)	(13.6)	(15.2)	(14.4)	–[1]
Diameter of decapsulated cysts	234.1	228.0	215.1	218.9	–
standard deviation	(11.7)	(12.2)	(12.9)	(11.9)	–
Chorion thickness	12.4	11.8	10.2	10.4	–

[1] No data available.

Table III shows high hatching percentages for the samples from solar saltworks. Maybe that the cysts collected from beaches of the salt lakes have been exposed to suboptimal conditions, *e.g.* repeated hydration-dehydration cycles or too long exposure to sunlight (Sorgeloos *et al.*, 1976 ; Vanhaecke and Sorgeloos, 1982) which can result in mortality of a part of the embryos.

TABLE III

Hatching characteristics of *Artemia* cysts form Tunisia

	Mégrine	Bekalta	Sfax 218	Sfax 117	Moknine	Sidi el Hani
Hatching percentage	60.5	83.2	84.8	76.0	14.2	43.1
Hatching efficiency (in nauplii/g product)	169 173	229 920	305 120	187 413	23 040	–
Hatching rate						
T_0	15.2	16.0	14.0	–	–	–
T_{50}	24.3	25.8	22.6	–	–	–
T_{90}	39.8	36.2	32.8	–	–	–
T_s	22.0	15.8	16.2	–	–	–

T_0 : time until appearance of first nauplii.

T_{50} : time until 50 % hatching is attained.

T_{90} = time until 90 % hatching is attained.

$T_s = T_{90} - T_{10}$. T_s is a measure for hatching synchrony.

A good illustration of the importance of pre-harvest conditions can be given for the cysts collected from the Sfax salterns : *i.e.* the hatching percent of the Sfax 218 sample, harvested from the brine, exceeds the value of the Sfax 117 cysts which were collected from the shore, by almost 10 %. The even larger difference in hatching efficiency reflects the risks of higher amounts of impurities, *e.g.* sand, when collecting the cysts from the shore. Despite a similar hatching percent value for the Bekalta and Sfax samples, the hatching efficiency of the latter is significantly higher. This can be attributed to the smaller size of the Sfax cysts and the purity of the sample.

The slow hatching rate and poor hatching synchrony observed in all samples (Table III, Fig. 2) is a drawback for their optimal use in aquaculture hatcheries. At the time of harvest (T_{90}) already part of the nauplii will have molted into the instar II and III stage which have lost a considerable amount of energy in comparison with the instar I stage (Benijts *et al.*, 1976 ; Vanhaecke *et al.*, 1983). As a consequence good yields of instar I nauplii can only be obtained when applying a two-step harvest, re-incubating the unhatched cysts after a first harvest of nauplii for example at T_{50}.

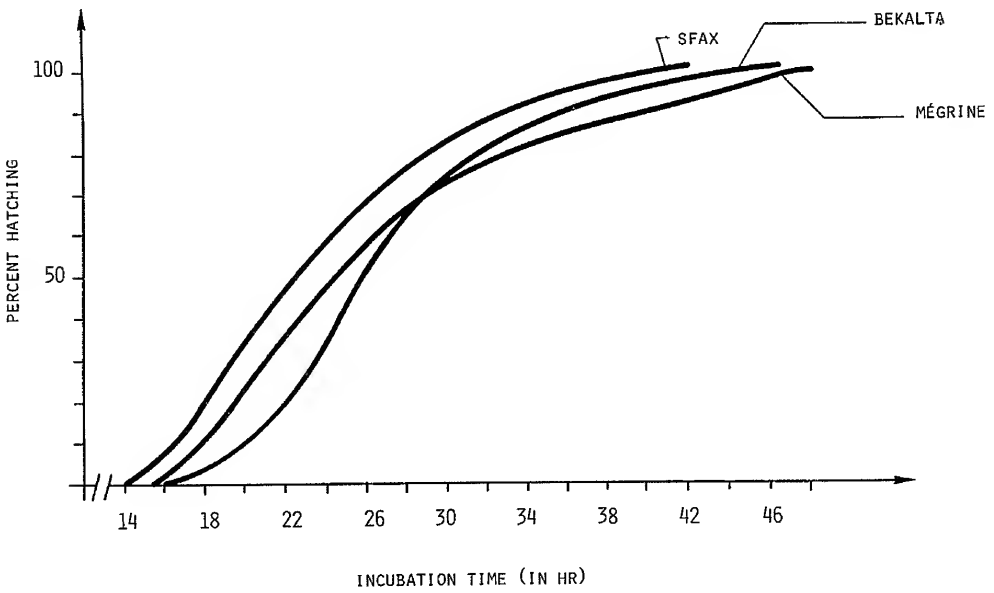

FIG. 2. Hatching curves for different *Artemia* cyst sources from Tunisia.

With the exception of the Sfax nauplii, which approximate the size range of San Francisco Bay nauplii (Vanhaecke and Sorgeloos, 1980b), the Tunisian *Artemia* produce rather large nauplii with a correspondent high dry weight (Fig. 3). Nevertheless sizes are still significantly smaller than the values obtained for parthenogenetic *Artemia*. They approximate the value for Great Salt Lake nauplii (Vanhaecke and Sorgeloos, 1980b) which means that only the smallest fish larvae might have ingestion problems with Tunisian *Artemia* (Beck and Bengtson, 1981).

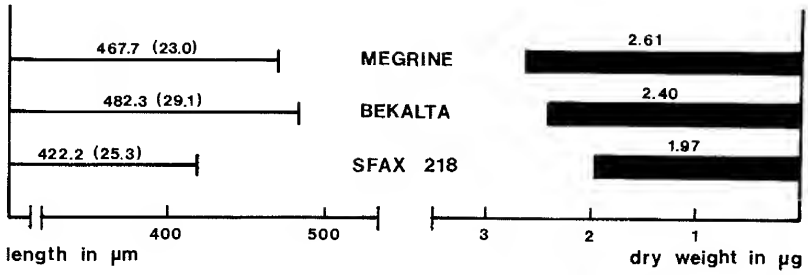

FIG. 3. Length and dry weight of instar I *Artemia* nauplii from various locations in Tunisia (standard deviations in parentheses).

Whereas Mégrine *Artemia* contain high levels of the HUFA 20:5ω3 (Table IV), Sfax nauplii contain only 3.1 mg/g which is marginally acceptable for a good *Artemia* diet for marine predator larvae (Léger *et al.*, 1986). As in most other *Artemia* strains no 22:6ω3 was detected in Tunisian *Artemia* (Léger *et al.*, 1986). The nutritional experiments with mysid shrimp reveal that the two Tunisian *Artemia* sources are an adequate diet (Table V ; Fig. 4). Survival, biomass production and reproduction activity of the shrimp fed the Tunisian *Artemia* are similar to the data obtained with nutritionally good Reference *Artemia*, and are significantly higher than the data obtained for Great Salt Lake, North Arm *Artemia*, known to be of inferior quality (Léger *et al.*, 1986). The good results with the Sfax strain, although low in 20:5ω3, is not surprising. The HUFA profile in Sfax *Artemia* is comparable to that found for Great Salt Lake South Arm cysts (Schauer *et al.*, 1980) which ensured good results with mysids (Johns *et al.*, 1981) but caused total mortality with brachyuran crab larvae (Johns *et al.*, 1980) and poor growth and survival in *Penaeus vannamei* and *P. stylirostris* (Léger *et al.*, 1986). As indicated by the latter authors, a 20:5ω3 content between 3 % and 4 % of total fatty acid methyl esters (*i.e.* 3 to 4 mg/g in instar I nauplii) seems to represent a marginal value of which the acceptability by different predators is not yet fully understood. It would therefore be incautious to designate the Sfax sample as a good quality product without additional culture data for other marine predators.

TABLE IV

Fatty acid methyl esters in freshly-hatched *Artemia* ;
expressed as mg fatty acid methyl ester per g *Artemia* dry weight
(GSLNA = Great Salt Lake, North Arm ; RAC = Reference *Artemia* cysts)

	Mégrine	Sfax 218	GSLNA	RAC
18:2ω6	6.3	5.3	6.0	6.2
18:3ω6	0.5	0.5	0.5	0.6
18:3ω3	15.6	17.4	18.0	3.0
20:4ω3	1.0	0.4	0.5	—[1]
20:5ω3	7.3	3.1	0.2	10.6
22:6ω3	—	—	—	—

[1] Undetectable.

TABLE V

Survival, dry weight, length and reproductive characteristics of *Mysidopsis bahia* cultured with *Artemia* nauplii from different cyst sources (GSLNA = Great Salt Lake, North Arm ; RAC = Reference *Artemia* Cysts)

	Mégrine	Sfax 218	GSLNA	RAC
Survival (%)	89.7[a]	94.7[a]	56.7[b]	93.2[a]
Standard deviation	(12.0)	(12.3)	(4.3)	(12.0)
Dry weight (µg)	319.3[ab]	330.1[a]	214.8[c]	280.7[ab]
Standard deviation	(48.0)	(20.0)	(34.9)	(40.9)
Length (µm)	5 209[a]	5 287[a]	4 321[c]	4 832[b]
Standard deviation	(105)	(219)	(273)	(169)
Reproductive characteristics[1] :				
sexually differentiated animals	100.0	98.1	89.3	100.0
$♀_i$	–	–	75.0	–
$♀_*$	82.4	74.8	25.0	80.0
$♀_m$	17.6	25.9	–	20.0

[abc] Means with different superscript are significantly different ($\alpha = 0.05$).

[1] Data in percent.

$♀_i$ = immature females.

$♀_*$ = females with eggs in ovaria.

$♀_m$ = females with eggs in marsupium.

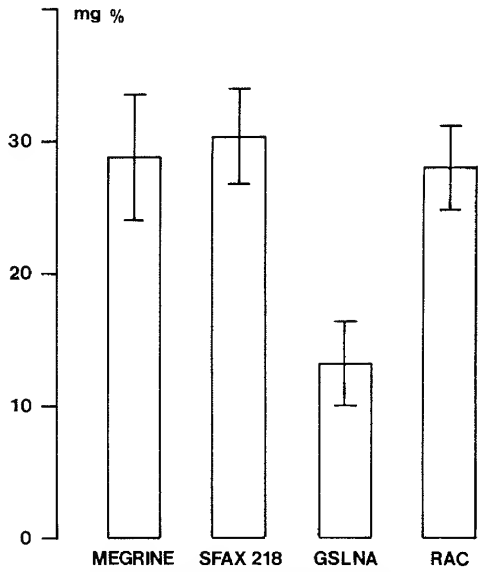

FIG. 4. Biomass production (expressed as mg %) of *Mysidopsis bahia* larvae cultured with *Artemia* nauplii from different cyst sources (GSLNA = Great Salt Lake, North Arm ; RAC = Reference *Artemia* Cysts).

Growth and survival data of Tunisian *Artemia* larvae in a standard culture test are given in Table VI. The average larval length for the reference strain after 7 days culture was 3.44 mm ± 0.18 mm at a survival of 90 %. Survival rates of the Tunisian *Artemia* are not significantly different from the reference strain. *Artemia* from Bekalta and Sfax grow significantly faster, respectively slower than the SFB reference strain. As larval growth rate is a strain specific characteristic (Vanhaecke and Sorgeloos, 1980a), Bekalta *Artemia* should be selected for biomass production purposes, yielding respectively 13 % and 21 % more biomass than the Mégrine and Sfax strains within the same period of time.

TABLE VI

Survival and growth of different *Artemia* strains from Tunisia in a standard culture test

	Mégrine	Bekalta	Sfax 218	Moknine	Sidi el Hani
Survival at day 7 (%)	85	88 86[2]	95	85	88
Growth[1]	100[b3]	115[a] 112[2]	85[c]	97[b]	103[b]

[1] Expressed as % of growth recorded for San Francisco Bay 288-2596.
[2] Result of replicate test in time.
[3] Means with different superscript are significantly different ($\alpha = 0.05$).

Fig. 5 reveals the poor tolerance to high temperature of Tunisian *Artemia*. This confirms the findings of Thoeye *et al.* (1987) who submitted Tunisian *Artemia* to diurnal temperature cycles. Since the GSL reference strain in our tests only yields 78 % and 32 % survival at respectively 30 °C and 34 °C, while in previous tests survival data of 90 % and 70 % respectively were recorded (Vanhaecke *et al.*, 1984), we suspect that our culture conditions, maybe the quality of the *Dunaliella*, were not optimal. Nevertheless the results show that the three indigenous strains already suffer considerable mortalities at a temperature of 30 °C.

This experiment provides interesting confirmation of limited temperature resistance of Tunisian *Artemia* of which the natural populations disappear during early summer when water temperatures exceed 30 °C. It should be mentioned that besides low temperature resistance of local strains, the production potential of *Artemia* in several saltworks is further limited by the presence of fish (*Aphanius* sp.) predating on *Artemia* at salinities as high as 150 ‰.

Conclusions

In conclusion it may be recognized that, although open for improvement, the overall quality of Tunisian *Artemia* is good, both in terms of hatching characteristics and nutritional effectiveness. Consequently, they can be used as an acceptable food source in aquaculture hatcheries. Furthermore, in view of their good growth rate, local strains can be used for intensive biomass production. Extensive culture might be limited, because of the poor resistance of local strains to high temperatures. Additional work is needed to assess the potential of the region for extensive cyst and biomass production.

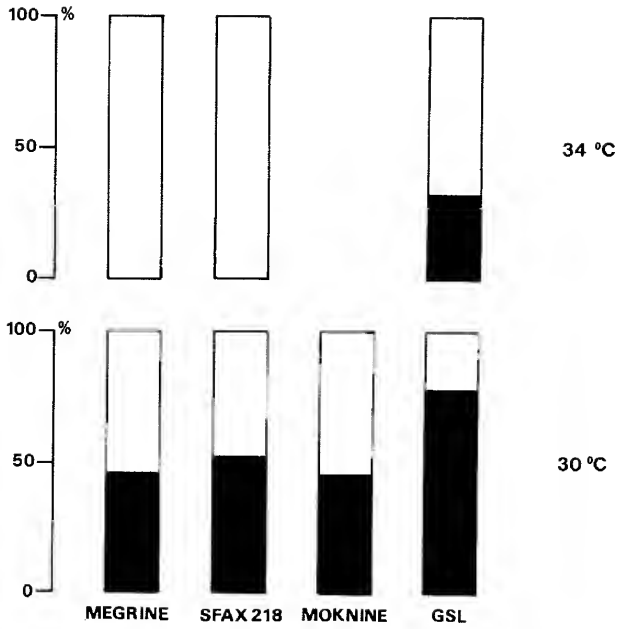

FIG. 5. Percent survival (black bars) of *Artemia* larvae from various origin reared at 30 °C and 34 °C in a standardized culture test (GSL = Great Salt Lake batch 1977).

Acknowledgements

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